

**WHOLE EXOME SEQUENCING GIVES ADDITIONAL BENEFITS COMPARED TO CANDIDATE GENE  
SEQUENCING IN THE MOLECULAR DIAGNOSIS OF CHILDREN WITH GROWTH HORMONE OR IGF-1  
INSENSITIVITY.**

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## 28 ABSTRACT

29

30 GH insensitivity (GHI) is characterised by short stature, IGF-1 deficiency and normal/elevated serum  
31 GH. IGF-1 insensitivity results in pre- and post-natal growth failure with normal/high IGF-1 levels.  
32 The prevalence of genetic defects is unknown.

33 **Objective:** To identify the underlying genetic diagnoses in a paediatric cohort with GH or IGF-1  
34 insensitivity using candidate gene (CGS) and whole exome sequencing (WES) and assess factors  
35 associated with the discovery of a genetic defect.

36 **Methods:** We undertook a prospective study of 132 patients with short stature and suspected GH or  
37 IGF-1 insensitivity referred to our centre for genetic analysis. 107 (96 GHI, 88 probands; 11 IGF-1  
38 insensitivity, 9 probands) underwent CGS. WES was performed in those with no defined genetic  
39 aetiology following CGS.

40 **Results:** A genetic diagnosis was discovered 38/107 (36%) patients (32% probands) by CGS. WES  
41 revealed 11 patients with genetic variants in genes known to cause short stature. A further 2  
42 patients had hypomethylation in the H19/IGF2 region or mUPD7 consistent with Silver-Russell  
43 Syndrome (total with genetic diagnosis 51/107, 48% or 41/97, 42% probands). WES also identified  
44 homozygous putative variants in *FANCA* and *PHKB* in 2 patients. Low height SDS and consanguinity  
45 was highly predictive for identifying a genetic defect.

46 **Conclusions:** Comprehensive genetic testing confirms the genetic heterogeneity of GH/IGF-1  
47 insensitivity and successfully identified the genetic aetiology in a significant proportion of cases. WES  
48 is rapid and may isolate genetic variants that have been missed by traditional clinically driven  
49 genetic testing. This emphasises the benefits of specialist diagnostic centres.

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## 55 INTRODUCTION

56

57 Short stature is one of the most common reasons for referral to paediatric endocrinologists. Patients  
58 with defects in growth hormone (GH) action or GH insensitivity (GHI) frequently present with severe  
59 phenotypes (height SDS  $\leq -2.5$ ) and the aetiology often remains uncertain. Consequently, many  
60 patients are classified as having idiopathic short stature (ISS) and pose a significant diagnostic and  
61 management challenge.

62

63 The growth hormone-insulin-like growth factor-1 (GH-IGF-1) axis is essential for human growth<sup>1</sup>. The  
64 cardinal features of GHI are severe growth failure, normal GH secretion and IGF-1 deficiency (IGFD).  
65 Monogenic defects leading to GHI have been discovered in *GHR*<sup>2, 3</sup>, *STAT5B*<sup>4, 5</sup>, *IGFALS*<sup>6</sup>, *PAPPA2*<sup>7</sup> and  
66 *IGF1*<sup>8</sup>. IGF-1 insensitivity secondary to *IGF1R* gene mutations exists as part of the continuum and  
67 leads to a similar phenotype<sup>9</sup>. In cases of IGF-1 resistance, the IGF-1 levels are high<sup>10</sup>. Depending on  
68 the genetic defect, associated clinical and dysmorphic features may be present including: mid-facial  
69 hypoplasia and frontal bossing (*GHR*, *STAT5B*)<sup>4, 11</sup>, immune deficiency (*STAT5B*)<sup>4</sup>, pubertal delay  
70 (*IGFALS*, *STAT5B*, *GHR*)<sup>4, 11, 12</sup>, decreased bone mineral density (*PAPPA2*)<sup>7</sup>, developmental delay,  
71 microcephaly and *in-utero* growth retardation (*IGF1*, *IGF1R*)<sup>1</sup>. 3M, Silver-Russell (SRS) and Noonan  
72 (NS) syndromes have phenotypes that can overlap with GHI<sup>10, 13, 14</sup>. 3M syndrome (OMIM 273750)  
73 results in pre- and post-natal growth restriction, prominent heels, facial dysmorphism and distinct  
74 radiological features<sup>15</sup>. The genetics are incompletely understood, but mutations in cullin 7 (*CUL7*)  
75 (70%), obscurin-like 1 (*OBSL1*) (25%) and coiled coil domain-containing 8 (*CCDC8*) (5%) genes have  
76 been identified<sup>16, 17</sup>. SRS is characterized by intrauterine and/or postnatal growth retardation and is  
77 caused by maternal uniparental disomy of chromosome seven (matUPD7) and hypomethylation of  
78 the imprinted H19/IGF2 domain of chromosome 11p15 in 10 and 35-65% cases, respectively<sup>18</sup>.  
79 Noonan syndrome results from autosomal dominant mutations in the Ras/mitogen activated protein  
80 kinase signalling pathways (*PTPN11*, *SOS1*, *SOS2*, *RAF1*, *BRAF*, *NRAS*, *KRAS*, *HRAS*, *CBL*, *RIT1*, *RASA2*,  
81 *MAP2K1*, *MAP2K2*, *A2ML1* *LZTR1* and *SHOC2* genes) in ~70% patients<sup>19, 20</sup>.

82

83 The identification of a pathogenic molecular defect is important for families and clinicians. A genetic  
84 diagnosis ends uncertainty, avoids unnecessary investigations and treatment, and allows  
85 appropriate genetic counselling and the identification of possible co-morbidities in syndromic short  
86 stature. A genetic diagnosis may also lead to earlier initiation of therapy and therefore a better long-  
87 term treatment response<sup>21</sup>.

88

89 Genetic defects can be identified by traditional Sanger sequencing of the most likely candidate genes  
90 (candidate gene sequencing, CGS) or by next generation sequencing e.g. whole exome sequencing  
91 (WES). CGS is clinically driven and is reliable when the affected gene can be predicted with a high  
92 degree of certainty. Its success depends on the accurate clinical phenotyping of patients and is  
93 limited in growth disorders with overlapping, highly variable or subtle features<sup>22</sup>. It is also time-  
94 consuming and costly if a number of genes are analysed. In contrast, WES allows the simultaneous  
95 screening of the entire coding DNA of an individual and is therefore extremely cost effective if  
96 multiple genes are to be investigated.

97

98 As a genetic reference centre, we undertook CGS in a cohort of patients with short stature and  
99 suspected GH or IGF-1 insensitivity. This is an extension of our previous work and some of the  
100 patients have been previously reported<sup>10</sup>. WES was completed in patients with no diagnosis  
101 following CGS. Our data demonstrate the importance of comprehensive genetic analysis in severe  
102 short stature, particularly the utility of WES in securing a molecular diagnosis where CGS has yielded  
103 negative results.

104

## 105 **SUBJECTS AND METHODS**

106

### 107 **Patients**

108 Between 2008 and 2017 our centre received 132 referrals (75M) for genetic investigation. Patients  
109 were referred from: UK (n=77), Kuwait (n=20), Poland (n=10), Germany (n=6), India (n=3), Thailand  
110 (n=3), Egypt (n=2), Argentina (n=2), Turkey (n=1), Italy (n=1), Mexico (n=1), Belgium (n=1), Denmark  
111 (n=1), Sweden (n=1), Croatia (n=1), UAE (n=1) and Ireland (n=1). Patients were investigated at their  
112 home institutions and the referring physicians completed a proforma detailing the clinical and  
113 biochemical data at the time of sending the DNA sample. The referring clinicians excluded causes of  
114 secondary GHI, including undernutrition. Birth weight, height, and BMI were expressed as SDS  
115 according to the appropriate national standards. Biochemical investigations included: basal and/or  
116 peak GH and basal IGF-1 levels. IGF-1 was expressed as SDS based on the age and sex appropriate  
117 range provided by the institution. Where serum IGF-1 was undetectable (less than the lower limit of  
118 the assay) (n=17), we calculated the lowest possible detectable SDS and assigned that for the  
119 statistical analysis. In these patients, the IGF-1 SDS ranged between -2.5 and -5.3 but this is likely to  
120 underestimate the degree of IGF-1 insensitivity.

121

122 Twenty-five of 132 patients did not have the clinical and biochemical characteristics of GH or IGF-1  
123 insensitivity and were excluded (**Figure 1A**). Diagnoses in the excluded group included GHD (n=4),  
124 short stature associated with chromosome 10 duplication (n=1) and achondroplasia (*FGFR3*  
125 mutation) (n=1). One hundred and seven cases (97 families, 97 probands) were investigated,  
126 including 49 patients (42 probands) with consanguineous parents. Ninety-six cases (58M, median  
127 age 5.8 years, range 0.1-17.0) had features of GHI: mean height -4.5 SDS (range -9.1 to -2.0), mean  
128 IGF1 -2.9 SDS (range -8.2 to -2.0) and peak GH levels 7-1195 µg/l. A further 11 children (2M, median  
129 age 5.8 years, range 0.1-14.4) had characteristics of IGF-1 insensitivity: mean height SDS -4.1 (range -  
130 6.8 to -2.4), mean birth weight SDS -3.1 (range -5.8 to -2.0), mean IGF-1 SDS 0.7 (range -1.1 to 4.4).

131

### 132 **Candidate gene sequencing (CGS)**

133 Genomic DNA was isolated from peripheral blood leukocytes (Qiagen DNeasy Kit) and genetic  
134 analysis was undertaken on all patients as previously described<sup>10</sup>. The candidate genes sequenced

depended on the clinical and biochemical features. Most patients were screened for mutations in the growth hormone receptor gene (*GHR*); other genes were selected depending on the phenotype (**Figure 1A**). Sanger sequencing was performed by the Barts and the London Genome Centre (<http://www.smd.qmul.ac.uk/gc/>) or GATC Biotech (<https://www.gatc-biotech.com>). 2 patients underwent molecular investigations for Silver-Russell syndrome (SRS) following referral to clinical geneticists. Three patients with GHI had *STAT5B* sequencing.

### **Whole Exome Sequencing (WES)**

WES was completed in 54 patients (53 probands and 11 unaffected relatives) who had no genetic cause for their short stature identified by CGS (**Figure 1A**). The remaining 15 patients did not consent for WES.

Twenty-three patients and 3 relatives were processed using the Agilent SureSelect all exon V4 capture and paired-end (2 x 100) sequencing on an Illumina HiSeq 2000 at Otogenetics (Norcross, GA). 31 patients and 8 relatives were sequenced using SureSelect Human All Exon v5 (51Mb) capture and paired-end (2 x 100) sequencing on an Illumina 2500 Standard run (minimum coverage 50x) at Oxford Gene Technology (OGT, Oxford, UK). >90% of target bases were covered 10X. For comparison, WES data from 43 in-house controls generated on the same platforms were analyzed by the same pipeline described below.

### **Variant analysis**

The raw data from Otogenetics or OGT were analysed using DNA Nexus (DNAnexus Inc., Mountain View, CA, USA) by aligning to the H. sapiens GRCh37–b37 (1000 genomes Phase 1) reference genome with BWA-MEM FastQ Readmapper VCF files, generated by Vendor Human Exome GATK-Lite Variant Caller (Unified Genotyper). The resulting VCF files were uploaded to Ingenuity Variant Analysis (Qiagen, Germany). The following filter settings were applied: call quality was set to  $\geq 20$  and read depth  $\geq 10$  and only data outside 0.1% of most exonically variable 100 base windows in healthy

public genomes and outside 0.1% most exonically variable genes in healthy public genomes (1000 genomes, ExAC) were included. Common variants were filtered out by excluding those with an allele frequency of  $\geq 0.1\%$  in the 1000 genomes, ExAC and the NHLBI exomes. Missense variations that were classified as loss of function by Ingenuity were included i.e. the amino acid change was predicted to affect function and those that were predicted benign were excluded. Variants that passed these filters and were predicted damaging by either SIFT or PolyPhen were explored further (Figure 1B):

*Analysis 1:* Variants were sought in 22 genes known to cause features of GHI or IGF-1 insensitivity (*GHR, IGFALS, STAT5B, IGF1, PAPP2, IGF1R, OSL1, CCDC8, CUL7, PTPN11, SOS1, SOS2, RAF1, BRAF, NRAS, KRAS, HRAS, CBL, RIT1, NF1, LZTR1* and *SHOC2*). Genetic variants were confirmed by Sanger sequencing (SS), primer sequences available on request. Forty-five family members underwent SS to assess the segregation of the variant within family structures. If no putative causal variants were found we progressed to analysis 2.

*Analysis 2:* Variants were sought in 153 biological candidate genes associated with: syndromic growth disorders, skeletal dysplasias, growth plate biology, cell proliferation, DNA repair or growth retardation in mice (Suppl. Table 1). An autosomal recessive model was adopted i.e. homozygous, hemizygous (for X-linkage) or potentially compound heterozygous variants as there were no affected parents. Variants were only included if they were present in patients and absent in controls. Since the cohort is genetically and phenotypically heterogeneous, we hypothesized that the same causal variant was unlikely to be seen in multiple patients (barring related individuals). Therefore, any variants that were present in  $\geq 3$  patients were discarded. If no putative causal variants were identified by these criteria we progressed to analysis 3.

*Analysis 3:* Variants were sought in novel candidate genes by an unbiased approach, seeking homozygous or putative compound heterozygous variants. Novel candidate genes were included if

189 predicted deleterious variants were identified in  $\geq 2$  patients and were absent in controls. Although  
190 this strategy may miss private mutations, it provides corroborative evidence that the gene is  
191 implicated in the phenotype. As above, identical variants that were present in  $\geq 3$  patients were  
192 discarded. Candidate genes satisfying these bioinformatic criteria were investigated *in silico* (see  
193 novel variants).

194

#### 195 **Rare variant burden testing**

196 Rare variant burden testing was applied to the pre-filtered variants from analysis 3 to identify genes  
197 enriched for rare variants in patients but not controls. The following script was employed using  
198 freeware R (<https://cran.r-project.org/>):

```
199         Table<-read.Table(file="genes.txt",head=FALSE) #imports file genes  
200         apply(Table,1, function(Table) fisher.test(matrix(Table,nr=2))$p.value)
```

201

#### 202 **Novel variants**

203 Novel variants were investigated *in silico* by SIFT (score ranges from 0 (predicted deleterious) to 1  
204 (predicted benign)), PolyPhen-2 (score ranges from 0 (predicted benign) to 1 (predicted  
205 deleterious)), Variant effect predictor (VEP), Mutation Taster and Human Splicing Finder (HSF  
206 version 3.0) to predict the functional outcome. VEP defines the likely deleterious effect of the  
207 variant as low, moderate, or high. Mutation Taster predicts whether the variant is predicted disease  
208 causing or benign. HSF predicts whether a variant makes exon skipping more likely than the  
209 reference allele. PubMed, OMIM and String determined Gene function and for pathway analysis.

210

#### 211 **Statistical analysis**

212 The differences in height SDS, IGF-1 SDS and peak GH between those with and without an identified  
213 genetic defect were analysed using an unpaired t-test. Univariate logistic regression analysis  
214 identified predictor variables (SPSS version 22; IBM Corp. Armonk, NY).



215

## 216 **Ethics**

217 Informed written consent for genetic research was obtained from patients and/or their parents.

218

## 219 **RESULTS**

220

### 221 **Diagnosis by Candidate Gene Sequencing (CGS)**

222 CGS identified likely causative variants in 35 GHI patients (28 probands) and 3 IGF-1 insensitivity  
223 patients, all probands (total 38/107; 36% or 31/97, 32% probands) (**Table 1**). These included variants  
224 in *GHR* (*n*=27 patients), *IGFALS* (*n*=3 patients), *OBSL1* (*n*=6 patients), *CUL7* (*n*=1 patients) and *IGF1R*  
225 (*n*=1 patient) (**Figures 1A and 2**). 30 of 38 (79%) children diagnosed by CGS had consanguineous  
226 parents. *STAT5B* sequencing was normal in the 3 patients tested.

227

228 *GHR*: Fifteen *GHR* variants (5 novel and 10 previously described) were identified in 27 patients  
229 (patients 1-27, **Table 1**) with mean serum IGF-1 -3.5 SDS (range -8.5 to -2.3), mean basal and peak  
230 GH concentrations 40.7 µg/l (range 1.8 to 398, *n*=21) and 123.2 µg/l (range 15.7–1195, *n*=20). All  
231 had homozygous variants in *GHR* with the exception of 2 patients (21 & 26) who had compound  
232 heterozygous variants, inheriting one defective allele from each parent. All children except patient 1  
233 had a “classical” Laron syndrome phenotype. The most commonly identified *GHR* defect was the  
234 homozygous *6ψ* mutation (c.618+792A>G, p.Met206\_Met207ins36) in patients 1-8 of UK Pakistani  
235 or Indian origin. These included two unrelated pairs of siblings (patients 2 & 3, 5 & 6) and four other  
236 non-familial cases (patients 1, 4, 7, 8). Patient 1 had a *GHR* intronic pseudoexon (*6ψ*) mutation with  
237 characteristic features of GHI (height SDS -4.0, IGF1 SDS -2.6, peak GH levels 119 µg/l) but no  
238 dysmorphic features. Four of the novel *GHR* variants were homozygous; c.198C>A (p.Cys66\*),  
239 c.700C>T (p.Gln234\*), c.599A>G (p.Asn200Ser) c.344A>C (p.Asn115Thr) all predicted deleterious by  
240 at least one functional outcome prediction method. Patient 21 had 2 previously described variants in  
241 compound heterozygosity (c.266+83G>T (p.?) and (c.723C>T, p.Gly241\_Glu261del). The final novel

variant, c.922G>A (p.Gly308Arg) predicted deleterious by SIFT, was found in compound heterozygosity with a known *GHR* variant in patient 26. Other known *GHR* mutations identified were: c.740T>C (p.Leu247Pro), c.594A>G (p.Glu198\*), c.785-6 T>A (p.Asp264Glyfx\*), c.247C>T (p.Gln83\*), c.703C>T (p.Arg235\*), c.439+1 G>A (p.Arg89Serfs\*47), c.723C>T (p.Gly241\_Glu261del) and c.168C>A (p.Cys56\*).

247

*IGFALS*: 3 GHI patients (28-30) had homozygous *IGFALS* variants (mean serum IGF1 SDS -2.7 (-3.6 to -1.9) and mean peak GH concentration 20.5 µg/L (16.0 to 28.9 µg/L). One variant c.1291delT, p.Trp431Glyfs\*11 has been previously described<sup>10</sup>. Interestingly, the previously described p.Leu134Gln variant identified in 2 patients (28 & 29) was associated with SGA but no dysmorphic features<sup>10</sup>.

253

*3M syndrome genes*: We identified 2 previously described homozygous *OBSL1* mutations c.1463C>T (p.Arg489\*) (patients 31 and 32) and c.1359insA, (p.Glu454Argfs\*) (patients 33-36) and 1 homozygous *CUL7* c.2710C>T (p.Arg904\*) mutation (patient 37)<sup>23, 24</sup>. All patients had consanguineous parents. All patients had severe short stature (mean height SDS -5.8) with normal GH (mean peak GH 21.8). Most had severe IGF-1 deficiency but 2 (patients 35 & 37) had IGF-1 levels of -0.2 and -0.25, respectively. Additional but variable clinical features of the 3M syndrome were present in all 7 patients (**Table 1**).

261

*IGF1R*: A heterozygous missense variant was identified in one patient with an IGF-1 insensitivity phenotype (birth weight -2.7 SDS, height SDS -3.1, IGF-1 SDS 2.0, basal GH 17.5 µg/l) (patient 38). This heterozygous variant, c.112G>A, (p.Asp38Asn) has previously been described (**Table 1**)<sup>10</sup>.

265

**Silver-Russell syndrome**

267 Hypomethylation in the imprinting control region 11p15 and mUPD7 was demonstrated in patients  
268 39 and 40, respectively. Both had features of GHI as previously described (frontal bossing, mid-facial  
269 hypoplasia, height SDS -3.7 and -4.3, and IGF-1 SDS -2.8 and -3.4) (**Figures 1A and Suppl. Table 2**)<sup>10</sup>.

270

271 **Diagnosis by Whole Exome Sequencing (WES) (Figure 1B)**

272 164,113 variants in 18,476 genes were called in 54 patient exomes (53 probands). Following the  
273 application of the filters described above for true rare predicted deleterious changes, this reduced to  
274 11,912 variants in 9,849 genes.

275

276 **Analysis 1 (Table 2 and Figure 2):** 11/54 patients (20%) (10 probands, 19%) were found to have  
277 variants in genes known to cause GHI (homozygous *GHR* (n=5), compound heterozygous *IGFALS*  
278 (n=1), homozygous *CCDC8* (n=1), homozygous *CUL7* (n=1), heterozygous *PTPN11* (n=2) and  
279 heterozygous *SOS1* (n=1)).

280

281 *GHR*: Patients 47-51 (**Table 2**) with *GHR* variants had classical Laron phenotypes (mean height SDS -  
282 5.1, mean IGF-1 SDS -4.7 and mean peak GH 46.8 µg/l). Patients 47-50 had a novel homozygous *GHR*  
283 variant c.70+4A>C (p.?) (exon skipping predicted by HSF) and were from consanguineous families of  
284 Kuwaiti origin, therefore a founder effect is likely. Patient 51 had a previously described *GHR*  
285 c.703C>T, p.Arg235\* (R217X) mutation, and had a clinical picture of classical Laron syndrome with  
286 height SDS -5.9, IGF-1 of -5.3, and peak GH >35.

287

288 *IGFALS*: Patient 46 (**Table 2**) with novel compound heterozygous *IGFALS* variants c.1576G>A  
289 (p.Asp526Asn) and c.632G>A, (p.Trp211\*), both predicted deleterious (SIFT score 0), had a typical  
290 phenotype (height SDS -5.0, IGF1 SDS -2.5 and peak GH 13 µg/L).

291

292 *Noonan syndrome (NS) genes*: Patients 41 and 42 had previously described heterozygous *PTPN11*  
293 c.417G>C (p.Glu139Asp) and c.853T>C (p.Phe285Leu) mutations<sup>25</sup>. Both had short stature (height

294 SDS -2.1 and -3.1), IGF-1 deficiency (-2.3 and -2.4), dysmorphic features and were SGA (birth weight  
295 SDS -2.1 and -3.0). The phenotype of the parents of patient 41 is unknown and we do not have  
296 parental DNA. The mother of patient 42 has the same variant and a clinical phenotype of NS. Patient  
297 43 had isolated short stature and a novel heterozygous c.3418T>A (p.Leu1140Ile) *SOS1* variant  
298 predicted disease causing by Mutation taster (**Table 2**). This patient's father also has a similar  
299 phenotype of isolated short stature (-2.4 SDS) but parental DNA was not available to confirm the  
300 segregation.

301

302 *3M syndrome genes*: Patients 44 and 45 had previously observed defects in *CCDC8* (c.612dupG,  
303 p.Lys205Glufs\*59) and *CUL7* (c.2988G>A, p.Trp996\*), respectively and had a classical GHI phenotype  
304 (**Table 2**).

305

306 *Analysis 2*: 43 remaining patients (all probands; 38 with GHI and 5 with IGF-1 insensitivity) were  
307 screened for variants in 153 biological candidate growth genes associated with: syndromic growth  
308 disorders, skeletal dysplasias, growth plate biology, cell proliferation, DNA repair or growth  
309 retardation in mice (**Suppl. Table 1**). A homozygous variant was identified in one patient in *FANCA*  
310 (c.2000C>G, p.P667R; mother heterozygous, paternal DNA not available) predicted damaging by SIFT  
311 and probably damaging by PolyPhen. A homozygous variant was identified in one patient in *PHKB*  
312 (c.56-1G>A; adopted child therefore parental DNA not available), which is associated with glycogen  
313 storage disease type IX (GSD IX). This alters one of the canonical splice site bases and is likely to  
314 cause exon skipping and an aberrant protein. 2 variants were identified in *MDC1*  
315 (c.3774\_3775delGCinsAT, p.P1259S and c.3528\_3529delGCinsAT, p.P1177S; both predicted  
316 tolerated/benign by PolyPhen and SIFT) in one patient and 2 variants in another patient in *EVC2*  
317 (c.673G>T, p.A225S; and c.664T>A, p.F222I; possibly and probably deleterious by PolyPhen,  
318 respectively) which were inherited together in cis from one parent who has normal stature. The  
319 *FANCA* and *PHKB* variants are potential candidates to explain the phenotype in 2 patients. In

contrast, due to the *in silico* predictions and mode of inheritance respectively, the *MDC1* and *EVC2* variants were presumed non-pathogenic.

*Analysis 3:* In light of the dearth of variants identified by analysis 2, WES data from all 43 undiagnosed patients were investigated using an unbiased approach. This strategy produced a shortlist of 109 variants in 77 candidate genes. Variants in all 77 genes were seen in GHI patients but only 4 genes had variants in patients with IGF-1 insensitivity (\*in **Supplementary Table 3**), none of which were specific to IGF-1 insensitivity. PubMed and OMIM did not reveal obvious growth associations of the 77 candidate genes and pathway analysis did not reveal any enriched functional pathways. On rare variant burden testing, none of the 77 candidate genes were found to be significantly enriched for deleterious variants in cases vs controls. Therefore, the significance of these variants is uncertain.

**Associations between phenotypic features and genetic defects**

Although there was significant overlap, patients with identified genetic defects were significantly shorter compared to those with no genetic diagnosis (mean height SDS -5.2 vs -3.7;  $p < 0.0001$ ) (**Figure 3**). Height SDS was significantly lower in patients with *GHR* or 3M gene mutations compared to individuals with no genetic diagnosis (both  $p < 0.0001$ ) (**Table 3**). IGF-1 SDS values were significantly lower in patients with any genetic defect and in those with *GHR* mutations compared to individuals with no genetic diagnosis ( $p = 0.0128$  and  $< 0.0001$ , respectively). GH levels were obtained from a number of different referral centres and likely measured by more than one assay. However, taking this limitation into account, peak GH levels were significantly higher in patients with *GHR* mutations compared to those with no genetic diagnosis ( $p = 0.0177$ ) (**Table 3**). Patients with *GHR 6Ψ* mutations had less severe phenotypes when compared to patients with other homozygous *GHR* defects (Mean height SDS -4.07 vs -6.2 respectively,  $p = 0.0006$ ) as previously described<sup>26</sup>. Consanguinity was predictive for identifying a molecular defect but age and sex were not (**Suppl. Table 4**).

347

## 348 **DISCUSSION**

349

350 In approximately 80% of patients with short stature the aetiology remains elusive despite detailed  
351 clinical, biochemical and radiological assessment<sup>27</sup>. This includes patients with extreme or syndromic  
352 short stature. Growth hormone (GHI) and IGF-1 insensitivity encompass a spectrum of clinical and  
353 biochemical abnormalities associated with normal GH secretion<sup>1</sup>. The degree of short stature is  
354 variable in this group of disorders but in many cases the growth failure is severe.

355

356 The majority of referrals to our genetic sequencing service were male as previously described<sup>10</sup>. Our  
357 cohort was heterogeneous but all patients had a phenotype consistent with GH or IGF-1 insensitivity  
358 i.e. short stature (height SDS  $\leq -2.0$ ), GH sufficiency (peak GH  $\geq 7.0$   $\mu\text{g/L}$ ) and low or normal/elevated  
359 IGF-1 levels, respectively.

360

361 Multiple mutations have been discovered in the GH-IGF-1 axis in association with GH and IGF-1  
362 insensitivity including mutations in the *GH1*, *GHR*, *STAT5B*, *IGFALS*, *PAPPA2*, *IGF1* and *IGF1R* genes<sup>1, 7</sup>.  
363 We recently noted that, as well as the classically recognised GH-IGF-1 axis gene defects, other short  
364 stature disorders may have features of GHI such as 3M, Noonan and Silver-Russell (SRS)  
365 syndromes<sup>10</sup>. Consequently, we now routinely screen GHI patients born small for gestational age  
366 (SGA) for mutations in the 3M syndrome genes (*OBSL1*, *CUL7* and *CCDC8*) as well as *IGF1*. Genetic  
367 testing for SRS was not carried out on other undiagnosed SGA subjects in this cohort; therefore, it is  
368 possible that other cases of SRS might have been missed. A proportion of short patients may carry  
369 disease-causing copy number variation (CNVs) or gene deletions / microdeletions<sup>28</sup> and analyses to  
370 detect this are currently underway in our undiagnosed patients. As such, deletions of candidate  
371 genes may have been missed by our analysis. Although detecting CNVs from WES is challenging, the  
372 use of algorithms may facilitate this process<sup>29</sup>.

373

374

375

376 Candidate gene sequencing (CGS) using Sanger sequencing is based on the selection of appropriate  
377 gene(s) for analysis depending on the patient's clinical phenotype and hormonal profile. This  
378 approach is reliant on accurate clinical information available at the time of referral and is usually  
379 restricted to a small number of genes due to the time and cost implications. In contrast, next  
380 generation sequencing techniques such as targeted gene panels can be employed to analyse all  
381 genes known to cause a genetically heterogeneous disorder in one test. Alternatively, whole exome  
382 sequencing (WES), allows the simultaneous analysis of all genes. Although gene panels can be  
383 powerful diagnostic tools, the advantage of WES is that data can also be mined for deleterious  
384 variants in novel genes not previously linked with a disease. Today, WES can be undertaken with a  
385 relatively low cost, however the interpretation of results can be difficult in inexperienced hands and  
386 the coverage of genes can be variable.

387

388 The traditional (CGS) approach alone confirmed a diagnosis in 35% of our cohort (31% probands);  
389 the majority of cases (92%) were diagnosed following sequencing of 1 or 2 genes. This technique is  
390 therefore relatively reliable if the phenotype is accurately documented and is typical for the disorder  
391 e.g. extreme short stature and IGF-1 deficiency (IGFD) with classical Laron syndrome features<sup>10, 30</sup>.  
392 Interestingly, we isolated a further 8 genetic variants in 11 patients in GHI genes by WES. These were  
393 not initially detected by CGS either because the variant was outside of the region amplified by  
394 Sanger sequencing in the case of the novel homozygous *GHR* gene mutation identified in 4 Kuwaiti  
395 patients or the phenotype was atypical (*IGFALS*, *PTPN11*, *SOS1*, *CCDC8*, *CUL7*). In the final Kuwaiti  
396 patient, the homozygous *GHR* mutation had been missed as a result of human error. Clinical  
397 phenotyping can be challenging for even experienced clinicians and many conditions have a wide  
398 phenotypic spectrum. In retrospect, the referring clinicians identified clinical features associated  
399 with Noonan and 3M syndromes in the 2 patients with previously reported *PTPN11* mutations and  
400 the patients with *CUL7* and *OBSL1* mutations, respectively. The patient with a novel heterozygous

401 *SOS1* gene variant was born SGA, had short stature and IGFD but no classical features of NS. The  
402 other patient with novel compound heterozygous *IGFALS* gene variants is shorter (-5.0 SDS) than  
403 most/all previously reported patients with IGFALS defects<sup>1</sup>. This emphasizes not only the importance  
404 of accurate clinical phenotyping prior to referral for genetic testing but also the difficulties in  
405 diagnosing many short stature syndromes. Noonan in particular, should be carefully considered  
406 when assessing a patient with features of GHI<sup>31</sup>.

407

408 Eleven novel genetic variants were identified in *GHR*, *IGFALS* and *SOS1* genes. As functional studies  
409 were not undertaken on the novel variants, it remains a possibility that they are not responsible for  
410 the clinical phenotype. However, familial segregation and *in silico* prediction programs have been  
411 utilized to substantiate them. Except for cases 42 (compound heterozygous *IGFALS*) and 45  
412 (heterozygous *SOS1*) the phenotypes are also typical for the identified genetic defects<sup>1</sup>. Therefore,  
413 we are confident that these genetic variants explain the clinical presentation. According to ExAC, the  
414 *SOS1* gene is intolerant of loss of function variants (expected number of loss of function variants  
415 57.5; observed loss of function variants 3, pLI = 1.0) this increases the likelihood of this variant being  
416 pathogenic. The *IGFALS* variants are both predicted to be highly deleterious and the patient had  
417 reduced birth weight (SDS -3.4). Together these factors may contribute to the development of a  
418 more extreme phenotype. No dysmorphic features or other potential genetic variants in candidate  
419 genes were identified in this patient that could explain the more severe phenotype. However, we  
420 cannot rule out oligogenicity with a novel gene defect. Prenatal growth retardation in particular has  
421 been previously recognized to contribute to the heterogeneity of IGFALS defects<sup>32</sup>.

422

423 We also identified a novel homozygous, predicted deleterious *FANCA* mutation in a patient with  
424 normal birth weight, short stature (-3.0 SDS) and IGFD (-2.0). Fanconi anaemia (FA), is an autosomal  
425 recessive trait, associated with skeletal and cardiac defects, pre- and post-natal growth retardation  
426 and malformation of the kidneys, although consistent with this case, 25% patients have no reported  
427 physical abnormalities<sup>33</sup>. The mean age at presentation is typical (usually ~7 yrs) and short stature is



428 recognised presenting feature in children<sup>33</sup>. Chromosome breakage test with mitomycin C (MMC)  
429 did not show any spontaneous chromosome fragility. Unfortunately, a lack of chromosomal fragility  
430 does not exclude FA and further investigations are currently underway. GSD IX is caused by *PHKB*  
431 mutations resulting in phosphorylase kinase deficiency. The novel, predicted damaging homozygous  
432 mutation was identified in a child with severe short stature (-4.5 SDS) and IGFD (-4.1 SDS). *PHKB* has  
433 an autosomal recessive mode of inheritance and the symptoms, severity and prognosis are highly  
434 variable, even among individuals with the same mutation. Characteristic features include,  
435 hepatomegaly, hypotonia, fasting hypoglycaemia and growth / pubertal delay. Although growth  
436 delays can be pronounced in affected children, catch-up growth is common and normal adult height  
437 is usually attained<sup>34</sup>. This patient is under investigation by the local metabolic team.

438

439 The identification of FA and GSD in children is crucial to initiate close monitoring for serious long-  
440 term complications (haematological malignancies / hepatic and cardiac, respectively) and studies are  
441 underway to validate these diagnoses. Ideally, functional studies should be undertaken to  
442 definitively attribute the *FANCA* and *PHKB* mutations to the phenotypes. Due to the *in silico*  
443 predictions and mode of inheritance respectively, the *MDC1* and *EVC2* variants were presumed non-  
444 pathogenic and have not been further investigated. The molecular diagnosis of all but 2 patients in  
445 the cohort could have potentially been secured using a next generation sequencing panel  
446 encompassing the genes included in Analysis 2. However, the advantage of WES is that it may  
447 serendipitously reveal a serious paediatric disorder, such as FA or GSD, which may have longer-term  
448 medical implications. Additionally, a gene panel would need to be continuously updated as further  
449 genetic causes of short stature are discovered. Furthermore, the cost of WES is significantly cheaper  
450 than undertaking CGS of the 22 genes known to cause GH and IGF-1 insensitivity (Analysis 1) i.e.  
451 approximately £600 vs £1750.

452

453 The identification novel genetic causes of short stature is essential to advance our understanding  
454 and management of growth disorders. To address this we used an unbiased approach (Analysis 3) to

455 uncover variants in genes, which might represent novel aetiologies for short stature. No strong  
456 candidate gene(s) emerged from this analysis but we hereby report the results for reference. The  
457 failure to identify other genes may be a result of wider genetic heterogeneity i.e. numerous  
458 undiscovered genes which make a major contribution to growth exist which cannot be identified in  
459 our relatively small cohort. Oligogenic inheritance of genes known to cause short stature may also  
460 explain some short stature phenotypes, although Analysis 2 does not support this. It is also possible  
461 that a combination of both these factors may be important. As we were unable to perform trio  
462 analysis on all patients, we may have missed some *de novo* variants acting in a dominant fashion.  
463 Additionally, CNV or unexplored e.g. intronic or regulatory regions of the known genes not covered  
464 by WES, such as the *GHR* pseudoexon mutation, may contribute <sup>26, 28</sup>. In the coming years, whole  
465 genome sequencing will uncover more such examples.

466

467 Deciding which short patients to refer for genetic testing can be problematic. Knowledge of the  
468 clinical features associated with different gene mutations is key to deciding which gene to prioritize.  
469 Our data suggests that accurate assessment of height, IGF-1 and GH may improve the diagnostic  
470 yield. Additionally, a genetic defect is more likely to be identified in consanguineous offspring. The  
471 current study suggests that CGS is reliable when the clinical features and the biochemical profile  
472 strongly suggest a particular candidate gene e.g. a *GHR* mutation. However, if a genetic diagnosis is  
473 not secured following sequencing of two candidate genes, then the CGS strategy is unlikely to reveal  
474 a genetic diagnosis and it is also more cost effective to proceed to WES.

475

476 We present the results of comprehensive genetic testing in a cohort of patients with GH and IGF-1  
477 insensitivity. A number of novel defects were identified in several genes associated with GH and IGF  
478 insensitivity. Our data expand the phenotypes associated with several genetic defects and also the  
479 spectrum of overlapping diagnoses associated with GHI. Next generation sequencing is an important  
480 adjuvant to CGS in the diagnosis of genetic short stature and emphasises the benefit of specialist  
481 diagnostic centres.

482 **URLs**

483 Ingenuity: <http://www.ingenuity.com/>  
484 freeware R: <https://cran.r-project.org/>  
485 HSF: <http://www.umd.be/HSF3/>  
486 Mutation taster: [www.mutationtaster.org](http://www.mutationtaster.org)  
487 VEP: [http://grch37.ensembl.org/Homo\\_sapiens/Tools/VEP](http://grch37.ensembl.org/Homo_sapiens/Tools/VEP)  
488 SIFT: <http://sift.jcvi.org>  
489 PolyPhen: <http://genetics.bwh.harvard.edu/pph2/>  
490 Pubmed: <https://www.ncbi.nlm.nih.gov/pubmed/>  
491 OMIM: <http://www.omim.org/>  
492 String: <http://string-db.org>  
493 ExAC: <http://exac.broadinstitute.org>  
494 HGVS: <http://varnomen.hgvs.org/>

495

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501

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503 HLS contributed to patient recruitment, data collection and analysis. SC performed the phenotypic  
504 and statistical analyses. HLS wrote the manuscript with input from SC, LAM, LS and DGR.

505

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507

508

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671 **FIGURE TITLES AND LEGENDS**

672

673 **Figure 1A. Summary of candidate gene (CGS) and whole exome sequencing (WES) in the GH and**  
674 **IGF-1 insensitivity patients.**

675

676 3M syndrome genes, *CUL7*, *CCDC8* and *OBSL1*; BW, birth weight.\*The candidate genes sequenced depended  
677 on the clinical and biochemical features. The majority of patients were screened for mutations in the growth  
678 hormone receptor gene (*GHR*) +/- *IGFALS*. Other genes were selected depending on the phenotype e.g.  
679 *STAT5B* if there was evidence of immune deficiency / eczema / atopy and *IGF1* and 3M genes if birth weight  
680 SDS was  $\leq 2.0$  SDS.

681

682 **Figure 1B. Whole exome sequencing (WES) data analyses: number of patients assessed and**  
683 **variants identified.**

684

685 **Figure 2. Genetic Diagnoses in the GH and IGF-1 insensitivity patients.**

686

687 **Figure 3. Height SDS in patients with a genetic diagnosis and those with no genetic diagnosis.**

688 Diagnosed patients n=50, undiagnosed n=55; ★ p = 0.0001

Table 1. Clinical, biochemical and genetic features of patients diagnosed by candidate gene sequencing (CGS) (total 37 patients, 39 variants)

Pt no.	Age (yr)	Sex	Consanguinity /ethnicity	Birth weight SDS	Height SDS	BMI SDS	Target Height SDS	GH basal (µg/L)	GH max (µg/L)	IGF-1 (ng/ml)	IGF-1 SDS	Clinical features	Genetic variants	MAF ExAC	Predicted outcome (novel variants)	Reference	No. genes analysed by CGS
<i>GHR</i> gene																	
1	8.0	M	+ /Pakistani	-0.5	-4.0	0.7	-0.9	13.2	119.0	18.2	-2.6	No	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
2 <sup>5</sup>	4.2	F	+ /Pakistani	0.1	-4.2	-1.0	0.7	16.3	33.3	<22.4	-2.5 <sup>3</sup>	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
3 <sup>5</sup>	7.5	M	+ /Pakistani	-2.9	-4.5	-1.2	-1.3	4.0	>33	1.4	-2.8	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
4	7.7	M	+ /Indian	-1.7	-3.1	-2.4	-1.9	3.2	30.3	11.2	-2.6	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
5 <sup>6</sup>	14.7	M	+ /Pakistani	0.7	-3.0	-0.7	-1.0	11.3	39.6	9.1	-3.1	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
6 <sup>6</sup>	2.3	M	+ /Pakistani	NK	-4.7	-0.5	-1.0	50.8	46.0	<22.4	-3.1 <sup>3</sup>	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
7	2.4	F	+ /Pakistani	-1.8	-5.0	-0.4	N/D	3.4	26.7	134.3	-2.3	Classical	Hom c.618+792A>G, p.Met206_Met207Ins36	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )
8	6.8	F	+ /Pakistani	-0.3	-4.1	-0.2	-0.9	56.1	30.3	30.3	-4.0	Classical	Hom c.618+792A>G, p.Met206_Met2	0	-	Metherell <i>et al</i> 2001 <sup>26</sup>	1 ( <i>GHR</i> )





3M syndrome genes																	
31	4.6	M	+/Bedouin	-3.2	-7.4	-0.7	-0.5	6.0	32	6.4	-2.5	Classical	<i>OBSL1</i> Hom c.1463C>T, p.Arg489* (p.R489X)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	2 ( <i>IGF1R</i> , <i>OBSL1</i> )
32	1.0	M	+/Kuwaiti	-1.6	-6.4	-2.3	-1.3	2.1	18.2	30.5	-2.5	Classical Hypermobility, prominent heels	<i>OBSL1</i> Hom c.1463C>T p.Arg489* (p.R489X)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	4 ( <i>GHR</i> , <i>IGFALS</i> , <i>IGF1</i> , <i>OBSL1</i> )
33	3.0	F	+/Kuwaiti	-5.2	-5.7	-4.7	NK	9.1	15	<3.0	-2.7 <sup>a</sup>	Classical Hypermobility, prominent heels	<i>OBSL1</i> Hom c.1359insA, p.Glu454Argfs* 11 (p.E454RfsX11)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	2 ( <i>IGF1</i> , <i>OBSL1</i> )
34 <sup>b</sup>	1.1	F	+/Kuwaiti	-3.8	-4.9	-0.4	-0.34	4.2	37.2	ND	ND	Classical. Hypermobility	<i>OBSL1</i> Hom c.1359insA, p.Glu454Argfs* 11(p.E454RfsX11)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	2 ( <i>IGF1</i> , <i>OBSL1</i> )
35 <sup>b</sup>	0.1	F	+/Kuwaiti	-2.6	-5.1	0.7	-0.34	5.4	10.8	105.0	-0.2	Classical Prominent heels	<i>OBSL1</i> Hom c.1359insA, p.Glu454Argfs* 11(p.E454RfsX11)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	2 ( <i>IGF1R</i> , <i>OBSL1</i> )
36	0.06	F	+/Kuwaiti	-1.5	-4.5	0.5	-1.2	41.0	33	<27	-2.6	Classical hypermobility, prominent heels short fingers, trident hands, short rib cage,	<i>OBSL1</i> Hom c.1359insA, p.E454Rfs*11 (p.E454RfsX11)	0	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	4 ( <i>GHR</i> , <i>IGFALS</i> , <i>IGF1</i> , <i>OBSL1</i> )



Table 2. Clinical, biochemical and genetic features of patients diagnosed by whole exome sequencing (WES) (total 11 patients, 12 variants)

Pt no.	Age (yr)	Sex	Consanguinity /ethnicity	Birth weight SDS	Height SDS	BMI SDS	Target Height SDS	GH basal (µg/L)	GH max (µg/L)	IGF-1 (ng/ml)	IGF-1 SDS	Clinical features	Genetic variants	MAF EXAC	Predicted outcome (novel variants)	Reference	No. genes analysed by CGS
Noonan syndrome genes																	
41	6.9	M	+/Kuwaiti	0.3	-2.1	-2.7	-1.5	1.1	>32	35.4	-2.3	Low set ears, undescended left testis	<i>PTPN11</i> Het c.417G>C p.Glu139Asp (p.E139D)	0	-	Tartaglia <i>et al</i> , 2002 <sup>25</sup>	1 ( <i>GHR</i> )
42	8.9	F	-/Polish	-2.1	-3.2	-1.6	0.6	21.7	10.5	47	-2.4	Low set ears, hypertelorism, mild ptosis, low posterior hairline	<i>PTPN11</i> Het c.853T>C, p.Phe285Leu (p.F285L)	0	-	Tartaglia <i>et al</i> , 2002 <sup>25</sup>	1 ( <i>GHR</i> )
43	13.1	M	-/Mexican-Russian	-3.0	-3.8	-1.5	-1.5	0.4	26.6	7.4	-2.63	No	<b><i>SOS1</i></b> Het c.3418T>A p.Leu1140Ile	3.5x E <sup>-5</sup> y	Disease causing (Mutation Taster)	Unpublished	2 ( <i>GHR</i> , <i>IGFALS</i> )
3M syndrome genes																	
44	1.9	F	+/Pakistani	-3.5	-5.7	-1.6	N/D	3.0	5.0	7.5	-1.8	Classical	<i>CCDC8</i> Hom c.612dupG, p.Lys205Glufs*59 (p.Lys205GlufsX 59)	1.8x E <sup>-5</sup> y	-	Hanson <i>et al</i> , 2009 <sup>23</sup>	1 ( <i>GHR</i> )
45	0.3	F	+/Kuwaiti	-5.8	-5.5	-0.6	NK	22.5	26.7	116	-1.1	Classical bilateral DDH	<i>CUL7</i> Hom c.2988G>A, p.Trp996* (p.W996X)	0	-	Al-Dosari <i>et al</i> , 2012 <sup>45</sup>	1 ( <i>GHR</i> )



													IGFALS gene				
46	15.4	F	+/ Pakistani	-3.4	-5.0	1.0	-3.4	0.1	13	35	-2.5	Classical	c.1576G>A p.Asp526Asn	1.7x E <sup>-4</sup> <sub>y</sub>	Deleterious (SIFT score 0)	Unpublish ed	1 (GHR)
													c.632G>A, p.Trp211*	0	Deleterious (SIFT score 0)	Unpublish ed	
													GHR gene				
47 <sup>h</sup>	3.5	F	+ / Kuwaiti	-0.82	-3.9	-1.2	-1.8	9.5	85	<30	-5.3	Classical	Hom c.70+4A>C, p.?	0	Variation predicted by HSF	Unpublish ed	1 (GHR)
48 <sup>h</sup>	2.2	F	+ / Kuwaiti	-0.34	-2.5	-0.4	1.8	22	ND	<30	-5.3	Classical	Hom c.70+4A>C, p.?	0	Variation predicted by HSF	Unpublish ed	1 (GHR)
49	2.0	F	+ / Kuwaiti	-2.1	-6.7	-2.3	0.3	35	>35	<30	-5.3	Classical	Hom c.70+4A>C, p.?	0	Variation predicted by HSF	Unpublish ed	1 (GHR)
50	1.2	F	+ / Kuwaiti	+0.12	-6.7	-1.3	-1.4	11.3	>32	<10	-2.1	Classical	Hom c.70+4A>C, p.?	0	Variation predicted by HSF	Unpublish ed	1 (GHR)
51	1.4	M	+ / Kuwaiti	+1.99	-5.9	-0.2	-0.1	>35	>35	<30	-5.3	Classical	Hom. c.703C>T, p.Arg235* (R217X)	8.2x E <sup>-6</sup> <sub>y</sub>	-	Amselem <i>et al</i> , 1993 <sup>39</sup>	1 (GHR)

Novel genetic variants are in bold font. Patients that underwent WES had no genetic diagnosis obtained following candidate gene sequencing. Height SDS is at presentation.

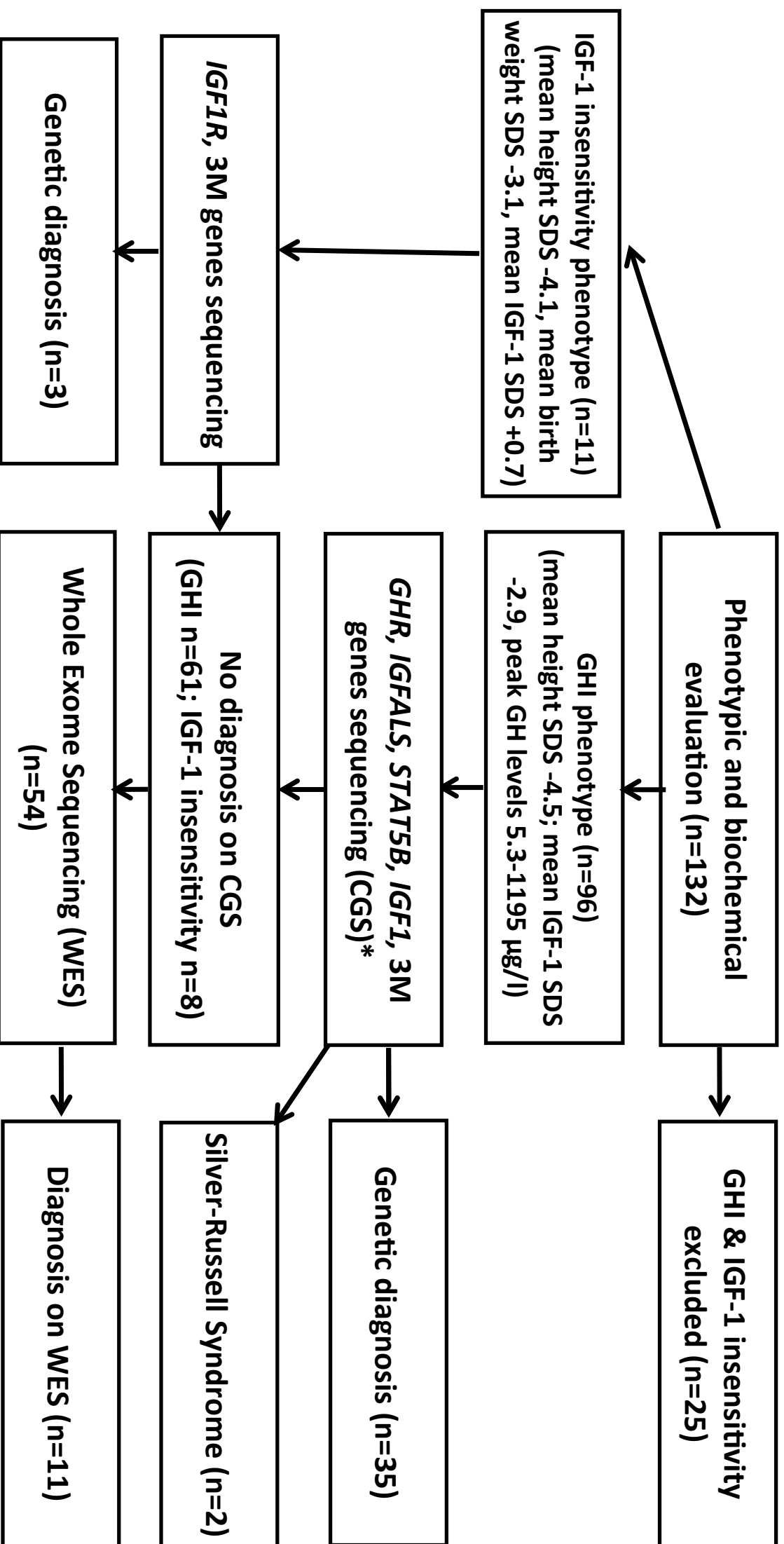
ND, not done; + parents consanguineous; -, parents not consanguineous; classical, classical GHI phenotype (frontal bossing, mid-facial hypoplasia); No, no dysmorphic features; DDH, developmental dysplasia of the hip; Hom, homozygous; Het, heterozygous; VEP, variant effect predictor; h, sisters. 3M syndrome genes, *CUL7*, *CCDC8* and *OBSL1*. Noonan syndrome genes, *PTPN11*, *SOS1*. c. coding DNA sequence where nucleotide 1 is the A of the ATG-translation initiation codon, for *GHR* the transcript includes exon 3 NCBI Reference Sequences NM\_000163; for *IGFALS* NM\_004970; for *CUL7* NM\_014780; for *CCDC8* NM\_032040; for *SOS1* NM\_005633; *PTPN11* NM\_001330437; ins, insertion; fs, frameshift; \*, termination site; as, acceptor site; ds, donor site; X, stop codon; del, deletion;<sup>β</sup> predicted result if exon is skipped. c. coding DNA sequence where

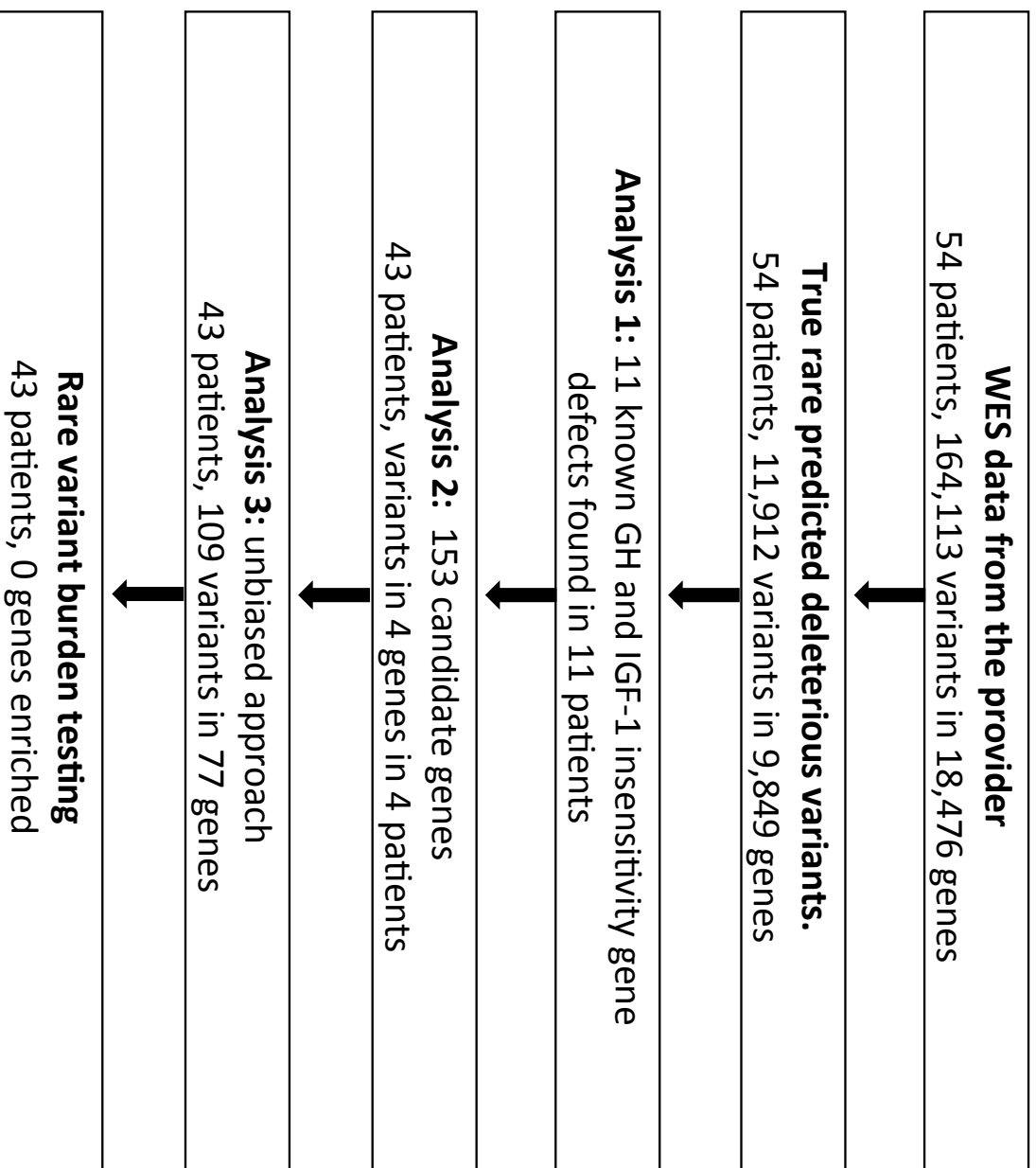
nucleotide 1 is the A of the ATG-translation initiation codon of *OBSL1* gene, NCBI reference NM\_015311.2; MAF, minor allele frequency - variants are defined as rare if the MAF is <0.001 (0.1%) as recorded on the ExAC (Exome Aggregation Consortium) database;  $\gamma$ , no homozygotes in ExAC database. Variation predicted by HSF, HSF predicts exon skipping to be more likely than in reference allele; SIFT score 0 is deleterious, 1 is benign. References refer to the genetic variants. No. genes sequenced by CGS, number of genes (and which genes) sequenced by candidate gene sequencing (CGS) before proceeding to whole exome sequencing (WES). Variant nomenclature is according to the HGVS guidelines.

**Table 3. Comparison of mean height SDS, IGF-1 SDS and peak GH levels between individuals with genetic defects and those with no molecular diagnosis**

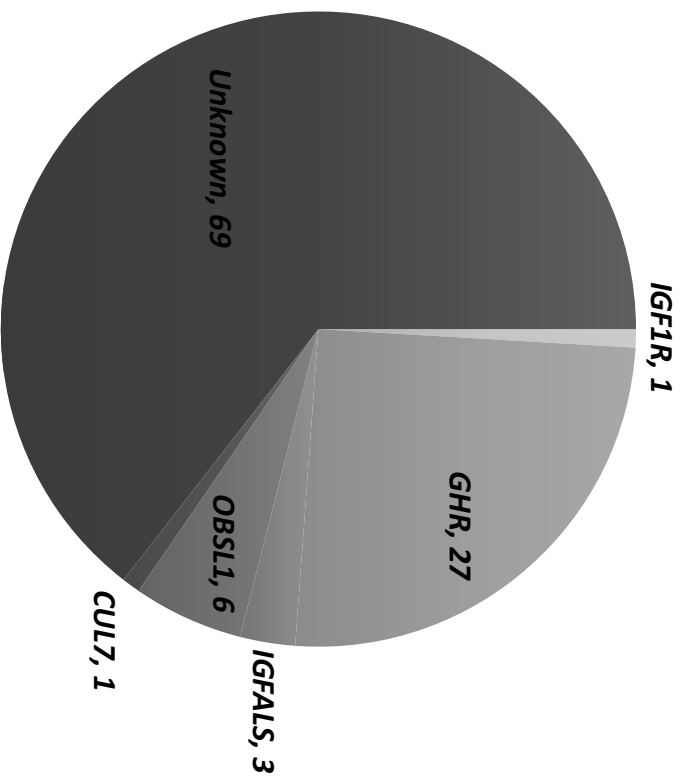
	No genetic diagnosis (Group 1)	<i>GHR</i> and <i>GHR</i> 6Ψ mutations (Group 2)	3M gene mutations (Group 3)	Any genetic diagnosis (Group 4)	Group 1 vs Group 2 P value (95% CI)	Group 1 vs Group 3 P value (95% CI)	Group 1 vs Group 4 P value (95% CI)
<b>Mean height SDS*</b>	-3.7 ± 1.2 (n=50)	-5.7 ± 1.9 (n=31)	-5.7 ± 0.9 (n=9)	-5.2 ± 1.8 (n=51)	<b>&lt;0.0001</b> (1.3 to 2.6)	<b>&lt;0.0001</b> (1.2 to 2.8)	<b>&lt;0.0001</b> (0.89 to 2.1)
<b>Mean IGF-1 SDS*</b>	-2.1 ± 1.5 (n=48)	-3.7 ± 1.9 (n=26)	-1.6 ± 1.0 (n=8)	-3.0 ± 2.0 (n=42)	<b>&lt;0.0001</b> (0.87 to 2.45)	0.3682 (-1.6 to 0.6)	<b>0.0128</b> (0.2 to 1.63)
<b>Mean peak GH*</b>	21.6 ± 17.1 (n=51)	93.7 ± 212.3 (n=31)	21.4 ± 13.4 (n=9)	68.1 ± 173.3 (n=48)	<b>0.0177</b> (12.9 to 131.3)	0.9676 (-12.3 to 11.8)	0.0594 (-1.88 to 94.9)

\*Means ± S.D.; CI, confidence intervals; 3M gene mutations, mutations identified in *CUL7*, *CCDC8* and *OBSL1*.



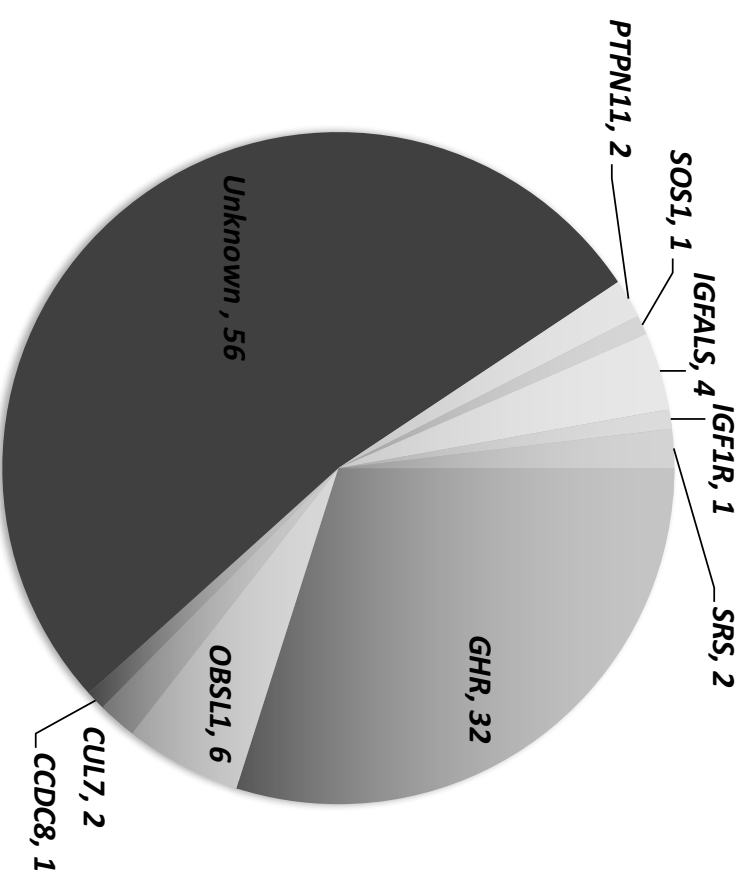


## CGS

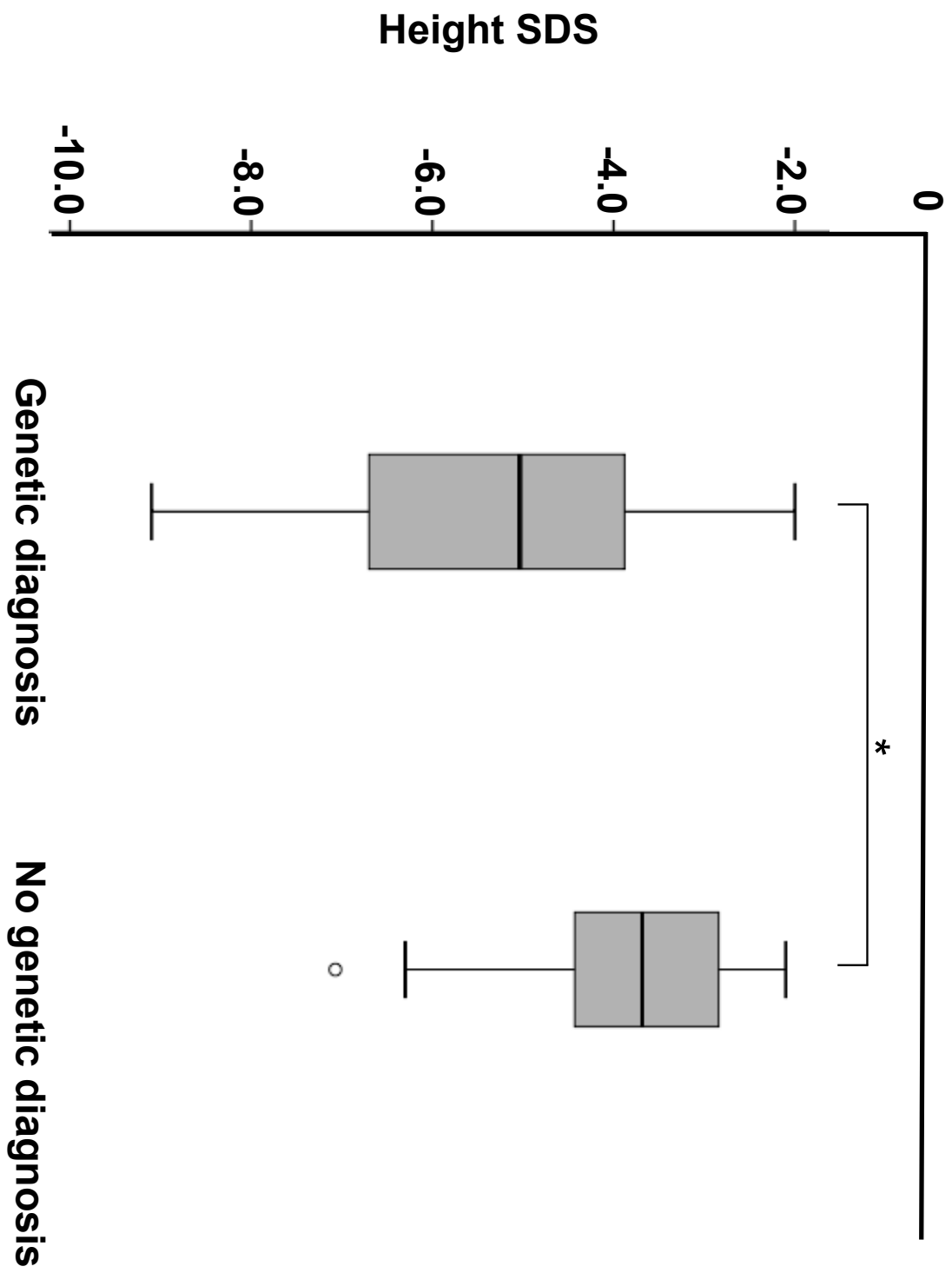


(38/107) 36%

## All modalities



(51/107) 48%



**Supplementary Table 1. List of biological candidate genes and their functional roles for genetic variant Analysis 2 (n=153)**

<b>Gene</b>	<b>Functional roles / associated disease</b>
<b><i>ADAM12</i></b>	ADAM metallopeptidase domain 12; Involved in skeletal muscle regeneration, specifically at the onset of cell fusion.
<b><i>ACAN</i></b>	AGGRECAN 1; Aggrecan is a major component of cartilage extracellular matrix. Defects are associated with short stature
<b><i>AGL</i></b>	amylo-alpha-1, 6-glucosidase, 4-alpha-glucanotransferase. Glycogen storage disease and associated growth retardation
<b><i>AKT1</i></b>	AKT1 is one of 3 closely related serine/threonine- protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis.
<b><i>AKT2</i></b>	v-akt murine thymoma viral oncogene homolog 2; AKT2 is one of 3 closely related serine/threonine- protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis.
<b><i>AKT3</i></b>	AKT3 is one of 3 closely related serine/threonine- protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis.
<b><i>ANKRD11</i></b>	ankyrin repeat domain 11; KBG syndrome and associated short stature
<b><i>ATR</i></b>	Acting as a DNA damage sensor. Recognizes the substrate consensus sequence [ST]-Q. Phosphorylates BRCA1, CHEK1, MCM2, RAD17, RPA2, SMC1 and p53/TP53, which collectively inhibit DNA replication and mitosis and promote DNA repair, recombination and apoptosis.
<b><i>ATRIP</i></b>	ATR interacting protein ATRIP is phosphorylated by ATR, regulates ATR expression, and is an essential component of the DNA damage checkpoint pathway
<b><i>B3GAT3</i></b>	Beta-1,3-glucuronyltransferase 3; Multiple joint dislocations, short stature, craniofacial dysmorphism, with or without congenital heart defects.
<b><i>B4GALT7</i></b>	Xylosylprotein 4-beta-galactosyltransferase, polypeptide 7; defects cause Ehlers-Danlos syndrome with short stature and limb anomalies.
<b><i>BLM</i></b>	Bloom syndrome, RecQ helicase-like; Participates in DNA replication and repair. Exhibits a magnesium-dependent ATP-dependent DNA-helicase activity that unwinds single- and double-stranded DNA in a 3'-5' direction. Involved in 5'-end resection of DNA during double-strand break (DSB) repair.
<b><i>BMP6</i></b>	bone morphogenetic protein 6; Induces cartilage and bone formation
<b><i>BOD1L1</i></b>	Biorientation of chromosomes in cell division 1-like 1; Recognition and repair of damaged replication forks is essential for maintenance of genome stability. BOD1L is a component of the fork protection pathway that responds to replication stress.
<b><i>CDC6</i></b>	Cell division cycle 6 homolog ( <i>S. cerevisiae</i> ); Involved in the initiation of DNA replication. Also participates in checkpoint controls that ensure DNA replication is completed before mitosis is initiated.



<b>CDK1</b>	Cyclin-dependent kinase 1; Plays a key role in the control of the eukaryotic cell cycle by modulating the centrosome cycle as well as mitotic onset; promotes G2-M transition, and regulates G1 progress and G1-S transition via association with multiple interphase cyclins.
<b>CDKN1B</b>	Cyclin-dependent kinase inhibitor. The HER2-HER3 dimer induces cell growth by activating a kinase cascade that includes phosphorylation of CDKN1B, resulting in CDKN1B ubiquitination and proteasomal degradation.
<b>CDKN1C</b>	Cyclin-dependent kinase inhibitor 1C (p57, Kip2); IMAGe syndrome - severe IUGR and marked postnatal growth failure.
<b>CDT1</b>	Chromatin licensing and DNA replication factor 1; Cooperates with CDC6 to promote the loading of the mini- chromosome maintenance complex onto chromatin to form the pre- replication complex necessary to initiate DNA replication. Meier-Gorlin syndrome 4 associated with short stature.
<b>CENPJ</b>	Centromere protein J; Plays an important role in cell division and centrosome function by participating in centriole duplication. Inhibits microtubule nucleation from the centrosome. Seckel syndrome 4 - intrauterine and postnatal growth retardation.
<b>CEP152</b>	Centrosomal protein 152kDa; Regulator of genomic integrity and cellular response to DNA damage acting through ATR-mediated checkpoint signaling. Necessary for centrosome duplication. It functions as a molecular scaffold facilitating the interaction of PLK4 and CENPJ, two molecules involved in centriole formation.
<b>CEP63</b>	Centrosomal protein 63kDa; Required for normal spindle assembly. Maintains centrosome numbers through centrosomal recruitment of CEP152. Also recruits CDK1 to centrosomes. Plays a role in DNA damage response. (By similarity).
<b>CHD7</b>	Chromodomain helicase DNA binding protein 7; Probable transcription regulator. CHARGE syndrome, retardation of growth.
<b>CHEK1</b>	Checkpoint kinase 1; Serine/threonine-protein kinase which is required for activation of DNA repair in response to the presence of DNA damage or unreplicated DNA.
<b>COL2A1</b>	Collagen, type II, alpha 1. Type II collagen, also called cartilage collagen, is the major collagen synthesized by chondrocytes.
<b>COL27A1</b>	Collagen, type xxvii, alpha-1; Steel syndrome: dislocated hips and radial heads, carpal coalition, scoliosis, and short stature.
<b>CREBBP</b>	CREB binding protein; Rubinstein-Taybi syndrome - growth retardation.
<b>DGCR8</b>	DiGeorge syndrome critical region gene 8.
<b>DLK1</b>	Delta-like 1 homolog (Drosophila); Phenotype is characterized by prenatal and postnatal growth retardation.
<b>DPH1</b>	<i>DPH1</i> , <i>S. cerevisiae</i> , homolog of; Developmental delay with short stature, dysmorphic features, and sparse hair.
<b>EGR1</b>	Early growth response 1; Transcriptional regulator. Recognizes and binds to the DNA sequence 5'-CGCCCCGC-3'(EGR-site). Activates the transcription of target genes whose products are required for mitogenesis and differentiation.

<b><i>EVC2</i></b>	Ellis van Creveld syndrome 2; Positive regulator of the hedgehog signalling pathway (By similarity). Plays a critical role in bone formation and skeletal development.
<b><i>FANCA</i></b>	Fanconi anemia, complementation group A; Clinical manifestations of Fanconi anemia include pre- and postnatal growth retardation; malformations of the kidneys, heart, and skeleton.
<b><i>FANCC</i></b>	Fanconi anemia, complementation group C; DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function. May be implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability.
<b><i>FANCD2</i></b>	Fanconi anemia, complementation group D2; Required for maintenance of chromosomal stability. Promotes accurate and efficient pairing of homologs during meiosis. Involved in the repair of DNA double-strand breaks, both by homologous recombination and single-strand annealing. May participate in S phase and G2 phase checkpoint activation upon DNA damage. Plays a role in preventing breakage and loss of missegregating chromatin at the end of cell division, particularly after replication stress.
<b><i>FANCG</i></b>	Fanconi anemia, complementation group G; DNA repair protein that may operate in a postreplication repair or a cell cycle checkpoint function. May be implicated in interstrand DNA cross-link repair and in the maintenance of normal chromosome stability.
<b><i>FBN1</i></b>	Fibrillin 1; Geleophysic Dysplasia 2 and Acromicric Dysplasia, both associated with short stature
<b><i>FBXW8</i></b>	F-box and WD repeat domain containing 8; Substrate-recognition component of a SCF-like E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins. In complex with CUL7, mediates ubiquitination and consequent degradation of GORASP1, acting as a component of the ubiquitin ligase pathway.
<b><i>FGD1</i></b>	FYVE, RhoGEF and PH domain containing 1; Aarskog-Scott syndrome associated with short stature.
<b><i>FGF8</i></b>	Fibroblast growth factor 8 (androgen-induced); Plays an important role in the regulation of embryonic development, cell proliferation, cell differentiation and cell migration.
<b><i>FGFR3</i></b>	Fibroblast growth factor receptor 3; Tyrosine-protein kinase that acts as cell-surface receptor for fibroblast growth factors and plays an essential role in the regulation of cell proliferation, differentiation and apoptosis. Plays an essential role in the regulation of chondrocyte differentiation, proliferation and apoptosis, and is required for normal skeleton development. Regulates both osteogenesis and postnatal bone mineralization by osteoblasts.
<b><i>FHL2</i></b>	Four and a half LIM domains 2; May function as a molecular transmitter linking various signalling pathways to transcriptional regulation. Negatively regulates the transcriptional repressor E4F1 and may function in cell growth.
<b><i>FOXRED1</i></b>	FAD-dependent oxidoreductase domain-containing protein 1; Leigh syndrome, mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b><i>G6PC</i></b>	Glucose-6-phosphatase, catalytic subunit; Glycogen storage disease (short stature is the feature in 90% patients) Hydrolyzes glucose-6-phosphate to glucose in the endoplasmic reticulum. Forms with the glucose-6-phosphate

	transporter (SLC37A4/G6PT) the complex responsible for glucose production through glycogenolysis and gluconeogenesis. Hence, it is the key enzyme in homeostatic regulation of blood glucose levels.
<b>GAA</b>	Glucosidase, alpha; acid; (short stature is the feature in 90% patients). Essential for the degradation of glycogen to glucose in lysosomes.
<b>GBA</b>	Glucosidase, beta, acid; Gaucher disease, includes subnormal growth velocity. Lysosomal storage disorder due to deficient activity of beta-glucocerebrosidase.
<b>GBE1</b>	Glucan (1,4-alpha-), branching enzyme 1; Required for sufficient glycogen accumulation. The alpha 1-6 branches of glycogen play an important role in increasing the solubility of the molecule and, consequently, in reducing the osmotic pressure within cells. Glycogen storage disease with stunted growth.
<b>GH1</b>	Growth Hormone
<b>GHRHR</b>	Growth hormone releasing hormone receptor; Receptor for GRF, coupled to G proteins which activate adenyl cyclase. Stimulates somatotroph cell growth, growth hormone gene transcription and growth hormone secretion.
<b>GHSR</b>	Growth hormone secretagogue receptor; Receptor for ghrelin, coupled to G-alpha-11 proteins. Stimulates growth hormone secretion. Binds also other growth hormone releasing peptides (GHRP) (e.g. Met-enkephalin and GHRP-6) as well as non-peptide, low molecular weight secretagogues (e.g. L-692,429, MK-0677, adenosine).
<b>GLI2</b>	GLI family zinc finger 2; acts as a transcriptional activator. May play a role during embryogenesis. Mutations cause Culler-Jones Syndrome which includes short stature secondary to hypopituitarism with growth hormone deficiency
<b>GLI3</b>	GLI-Kruppel family member 3; Pallister-Hall syndrome is a pleiotropic disorder comprising hypothalamic hamartoma, pituitary dysfunction, central polydactyly, and visceral malformations.
<b>NGEF</b>	Guanosine nucleotide exchange factor; Part of the RAS signalling pathway: Adaptor-GNEF complex translocates to the membrane where GNEF activates Ras.
<b>GRB10</b>	Growth factor receptor-bound protein 10.
<b>GRB2</b>	Growth factor receptor-bound protein 2; Adapter protein that provides a critical link between cell surface growth factor receptors and the Ras signaling pathway.
<b>GSC</b>	Goosecoid homeobox; Short stature, auditory canal atresia, mandibular hypoplasia, skeletal abnormalities
<b>GYS1</b>	Glycogen synthase 1 (muscle); Transfers the glycosyl residue from UDP-Glc to the non- reducing end of alpha-1,4-glucan. Glycogen storage disease.
<b>GYS2</b>	Glycogen synthase 2 (liver); Transfers the glycosyl residue from UDP-Glc to the non- reducing end of alpha-1,4-glucan. Glycogen storage disease.
<b>HESX1</b>	HESX homeobox 1; Required for the normal development of the forebrain, eyes and other anterior structures such as the olfactory placodes and pituitary gland. Growth hormone deficiency with pituitary anomalies.
<b>HMGA2</b>	High mobility group AT-hook 2; Functions as a transcriptional regulator. Functions in cell cycle regulation through CCNA2. Plays an important role in chromosome condensation during the meiotic G2/M transition of

	spermatocyte HMGA2 functioning is required for human growth and development.
<b>HRAS</b>	V-HA-RAS Harvey rat sarcoma viral oncogene homolog; Growth factor signalling pathway. Defects cause Costello syndrome is a rare multiple congenital anomaly syndrome associated in all cases with a characteristic coarse facies, short stature, distinctive hand posture and appearance, severe feeding difficulty, and failure to thrive.
<b>IGF2</b>	Insulin-like growth factor 2.
<b>IGF2R</b>	Insulin-like growth factor 2 receptor; This receptor binds IGF2, defects in which likely cause severe growth restriction with distinctive facies.
<b>IGFBP1</b>	Insulin-like growth factor binding protein 1; IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors. Promotes cell migration.
<b>IGFBP2</b>	Insulin-like growth factor binding protein 2, 36kDa; Inhibits IGF-mediated growth and developmental rates. IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors.
<b>IGFBP3</b>	Insulin-like growth factor binding protein 3; IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors. Also exhibits IGF-independent antiproliferative and apoptotic effects mediated by its receptor TMEM219/IGFBP-3R.
<b>IGFBP4</b>	Insulin-like growth factor binding protein 4; IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors.
<b>IGFBP5</b>	Insulin-like growth factor binding protein 5; IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors.
<b>IGFBP6</b>	Insulin-like growth factor binding protein 6; IGF-binding proteins prolong the half-life of the IGFs and have been shown to either inhibit or stimulate the growth promoting effects of the IGFs on cell culture. They alter the interaction of IGFs with their cell surface receptors.
<b>IRS1</b>	Insulin receptor substrate 1; May mediate the control of various cellular processes by insulin. IRS1 acts as an interface between signalling proteins with Src homology-2 domains and the receptors for insulin, IGF2, growth hormone, several interleukins, and other cytokines. Downregulation of IRS1 inhibited cell growth in HEK293 and breast cancer cells by suppressing cycle progression from G0/G1 to S phase CHICO, a Drosophila homolog of the vertebrate IRS gene family, plays an essential role in the control of cell size and growth.
<b>IRS2</b>	Insulin receptor substrate 2; Insulin receptor substrates (IRS proteins) mediate the pleiotropic effects of insulin and insulin-like growth factor-1, including regulation of glucose homeostasis and cell growth and survival. The experiments showed that Irs1 and Irs2 are critical for embryonic and

	postnatal growth, with <i>Irs1</i> having the predominant role.
<b>JAK2</b>	Janus kinase 2; Non-receptor tyrosine kinase involved in various processes such as cell growth, development, differentiation or histone modifications. Mediates essential signaling events in both innate and adaptive immunity. In the cytoplasm, plays a pivotal role in signal transduction via its association with type I receptors such as growth hormone (GHR).
<b>KAL1</b>	Kallmann syndrome 1 sequence; Kallmann syndrome has been reported to be associated with short stature.
<b>KDM5C</b>	Lysine-specific demethylase 5C; X-linked mental retardation and short stature, Claes-Jensen type
<b>KDM6A</b>	Lysine (K)-specific demethylase 6A; Kabuki syndrome. All of the patients with KDM6A mutations had short stature and postnatal growth retardation.
<b>LHX3</b>	LIM homeobox 3; A mouse recessive mutation called 'stubby' ( <i>stb</i> ) maps to the same area on chromosome 2 as the <i>Lhx3</i> gene. Homozygous <i>stb</i> mice exhibit disproportionate dwarfing, manifested in shorter than normal head, body, and legs. Patients with mutations in LHX3 were reported to have combined pituitary deficiency and skeletal abnormalities.
<b>LHX4</b>	LIM homeobox 4; 4 affected members of a French family with LHX4 mutation had short stature, pituitary and cerebellar defects, and abnormalities of the sella turcica of the central skull base.
<b>LIG1</b>	Ligase I, DNA, ATP-dependent; DNA ligase that seals nicks in double-stranded DNA during DNA replication, DNA recombination and DNA repair.
<b>LIG4</b>	Ligase IV, DNA, ATP-dependent; Efficiently joins single-strand breaks in a double-stranded polydeoxynucleotide in an ATP-dependent reaction. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination.
<b>LTBP3</b>	Latent transforming growth factor-beta-binding protein 3; associated with Dental anomalies and short stature
<b>MACROD2</b>	Macro domain-containing 2; Probably required for the association of ORC on chromatin during G1 to establish pre-replication complex (preRC) and to heterochromatic sites in post-replicated cells. Mutations cause Kabuki syndrome.
<b>MAP2K1</b>	Mitogen-activated protein kinase kinase; Growth factor signalling pathway.
<b>MASP1</b>	Mannan-binding lectin serine protease 1; Mutations cause 3MC syndrome: the main features of these syndromes are facial dysmorphism, cleft lip and palate, postnatal growth deficiency, cognitive impairment, and hearing loss.
<b>MCM10</b>	Minichromosome maintenance complex component 10; MCM10 mRNA level increased at the G1/S boundary when quiescent normal human fibroblasts were induced to proliferate with serum. MCM10 associated with nuclease-resistant nuclear structures throughout S phase and dissociated from them in G2 phase. MCM10 associated with ORC2 (ORC2L; 601182) when overexpressed in COS-1 cells, and it interacted with ORC2, MCM2 (116945), and MCM6 (601806) in a yeast 2-hybrid system.
<b>MCM2</b>	Minichromosome maintenance complex component 2; The MCM2-7 complex is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication
<b>MCM3</b>	Minichromosome maintenance complex component 3; The MCM2-7 complex

	is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication.
<b>MCM4</b>	Minichromosome maintenance complex component 4; The MCM2-7 complex is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication.
<b>MCM5</b>	Minichromosome maintenance complex component 5; The MCM2-7 complex is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication.
<b>MCM6</b>	Minichromosome maintenance complex component 6; The MCM2-7 complex is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication.
<b>MCM7</b>	Minichromosome maintenance complex component 7; The MCM2-7 complex is comprised of 6 subunits, MCM2 through MCM7, and is a ring-shaped heterohexameric ATPase involved in DNA replication.
<b>MCM8</b>	Minichromosome maintenance complex component 8; Absence of MCM8 in human U2OS cells reduced growth and homologous recombination (HR) efficiency under conditions of replication stress.
<b>MDC1</b>	Mediator of DNA-damage checkpoint 1. Mdc1 -/- mice were born at the expected mendelian frequency, but they showed a phenotype similar to that of H2ax -/- mice, including growth retardation, male infertility, immune defects, chromosome instability, DNA repair defects, and radiation sensitivity
<b>MLL2</b>	Myeloid/lymphoid or mixed lineage leukemia 2; Kabuki syndrome
<b>MMP14</b>	Matrix metalloproteinase 14; Winchester syndrome - syndrome characterized by short stature, severe joint contractures, peripheral corneal opacities, coarsened facies, dissolution of carpal and tarsal bones, and generalized osteoporosis.
<b>NBAS</b>	Neuroblastoma-amplified sequence; Short stature, optic nerve atrophy, and Pelger-Huet anomaly.
<b>NDUFAF2</b>	NADH dehydrogenase (ubiquinone) complex i, assembly factor 2 mitochondrial complex deficiency 1; Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b>NDUFAF3</b>	NADH dehydrogenase (ubiquinone) complex i, assembly factor 3; Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b>NDUFAF4</b>	NADH dehydrogenase (ubiquinone) complex i, assembly factor 4; mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b>NDUFB3</b>	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa; mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b>NDUFB9</b>	NADH-ubiquinone oxidoreductase 1 beta subcomplex, 9. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b>NDUFS1</b>	NADH-ubiquinone oxidoreductase Fe-S protein 1; Associated with a number

	of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b><i>NDUFS2</i></b>	NADH-ubiquinone oxidoreductase Fe-S protein 2; Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b><i>NDUFS4</i></b>	NADH-ubiquinone oxidoreductase Fe-S protein 4; mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans. Leigh syndrome.
<b><i>NDUFS6</i></b>	NADH-ubiquinone oxidoreductase Fe-S protein 6; Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans.
<b><i>NF1</i></b>	Neurofibromin 1; Stimulates the GTPase activity of Ras. NF1 shows greater affinity for Ras GAP, but lower specific activity. May be a regulator of Ras activity. Macrocephaly and short stature have been reported in several clinical studies of NF1.
<b><i>NHEJ1</i></b>	Nonhomologous end-joining factor 1; defects are known to cause severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation.
<b><i>NIPBL</i></b>	Nipped-b, drosophila, homolog of; Cornelia de Lange syndrome 1
<b><i>NPR2</i></b>	Natriuretic peptide receptor 2; defects are associated with short stature with nonspecific skeletal abnormalities.
<b><i>NUBPL</i></b>	Nucleotide-binding protein-like protein; mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans
<b><i>ORC1</i></b>	Origin recognition complex, subunit 1, s. Cerevisiae, homolog of; <i>ORC1</i> , a subunit of the origin recognition complex, is a key component of the DNA replication licensing machinery that also plays a role in controlling centriole and centrosome copy number in human cells independent of its role in DNA replication. Mutations cause Meier-Gorlin syndrome 1 -almost all cases have primordial dwarfism with substantial prenatal and postnatal growth retardation.
<b><i>OTX2</i></b>	Orthodenticle, drosophila, homolog of, 2; <i>OTX2</i> defects have been described to cause short stature and developmental delay.
<b><i>PAPPA</i></b>	Pregnancy-associated plasma protein A, pappalysin 1; Metalloproteinase which specifically cleaves IGFBP-4 and IGFBP-5, resulting in release of bound IGF. Cleavage of IGFBP-4 is dramatically enhanced by the presence of IGF, whereas cleavage of IGFBP-5 is slightly inhibited by the presence of IGF.
<b><i>PCNT</i></b>	Pericentrin; Microcephalic osteodysplastic primordial dwarfism, type II.
<b><i>PDCD4</i></b>	Programmed cell death 4; It's been proposed that regulated degradation of PDCD4 in response to mitogens allows efficient protein synthesis and consequently cell growth.
<b><i>PEG1</i></b>	Paternally expressed gene 1; The <i>PEG1</i> gene maps to an imprinted region of mouse chromosome 6 and is expressed monoallelically from the paternal allele. When the null allele is paternally transmitted, the offspring exhibits severe intrauterine growth retardation.

<b><i>PEG3</i></b>	Paternally expressed gene 3; The heterozygous mice that inherited the mutant allele from the paternal germline were smaller
<b><i>PEX2</i></b>	Peroxisomal biogenesis factor 2; Somewhat implicated in the biogenesis of peroxisomes. <i>Drosophila</i> pex mutants, including <i>PEX2</i> , faithfully recapitulated several key features of human peroxisome biogenesis disorder, including impaired peroxisomal protein import, elevated very long chain fatty acid (VLCFA) levels, and growth retardation
<b><i>PFKM</i></b>	Phosphofructokinase, muscle type; catalyzes the third step of glycolysis, the phosphorylation of fructose-6-phosphate (F6P) by ATP to generate fructose-1,6-bisphosphate (FBP) and ADP. Thought to regulate growth and metabolism
<b><i>PHKA1</i></b>	Phosphorylase kinase, alpha 1 (muscle); Phosphorylase b kinase catalyzes the phosphorylation of serine in certain substrates, including troponin I. Defects cause glycogen storage disease.
<b><i>PHKA2</i></b>	Phosphorylase kinase, alpha 2 (liver); Phosphorylase b kinase catalyzes the phosphorylation of serine in certain substrates, including troponin I. Defects cause glycogen storage disease.
<b><i>PHKB</i></b>	Phosphorylase kinase, beta; Phosphorylase b kinase catalyzes the phosphorylation of serine in certain substrates, including troponin I. The beta chain acts as a regulatory unit and modulates the activity of the holoenzyme in response to phosphorylation. Defects cause glycogen storage disease.
<b><i>PHKG2</i></b>	Phosphorylase kinase, testis/liver, gamma-2; known to cause growth retardation and glycogen storage disease.
<b><i>PIK3CA</i></b>	Phosphatidylinositol 3-kinase, catalytic, alpha, severe growth failure described in humans.
<b><i>PITX2</i></b>	Paired-like homeodomain transcription factor 2; transcription factor PITX2 is rapidly induced by the WNT/DVL/beta-catenin pathway and is required for effective cell type-specific proliferation by directly activating specific growth-regulating genes.
<b><i>PLAGL1</i></b>	Pleomorphic adenoma gene-like 1; <i>PLAGL1</i> knockdown in mice resulted in intrauterine growth restriction, altered bone formation, and neonatal lethality
<b><i>PLK4</i></b>	Polo-like kinase 4; Serine/threonine-protein kinase that plays a central role in centriole duplication. Defects cause growth retardation with dwarfism (up to -8 SD).
<b><i>POC1A</i></b>	POC1 centriolar protein, chlamydomonas, homolog of, a; short stature, onychodysplasia, facial dysmorphism, and hypotrichosis;
<b><i>POLE</i></b>	Polymerase, dna, epsilon; facial dysmorphism, immunodeficiency, livedo, and short stature.
<b><i>POU1F1</i></b>	POU class 1 homeobox 1; Transcription factor involved in the specification of the lactotrope, somatotrope, and thyrotrope phenotypes in the developing anterior pituitary. Activates growth hormone and prolactin genes.
<b><i>PPP1R15B</i></b>	Protein phosphatase 1, regulatory subunit 15B; Microcephaly, short stature, and impaired glucose metabolism 2.
<b><i>PRKAG2</i></b>	AMP-activated protein kinase, noncatalytic, gamma-2; glycogen storage disease.



<b><i>PROP1</i></b>	PROP paired-like homeobox 1; affected mice are of normal body size at birth but postnatal growth is severely retarded and the body size of adult animals is approximately one-third of normal.
<b><i>PYGL</i></b>	Glycogen phosphorylase, liver; short stature and glycogen storage disease.
<b><i>PYGM</i></b>	Glycogen phosphorylase, muscle; glycogen storage disease.
<b><i>RAB3IP</i></b>	RAB3A interacting protein (rabin3). Variants in RABIP have been reported in a patient with short stature.
<b><i>RIEG1</i></b>	Rieg bicoid-related homeobox transcription factor 1; transcription factor RIEG1 is rapidly induced by the WNT/DVL /beta-catenin pathway and is required for effective cell type-specific proliferation by directly activating specific growth-regulating genes.
<b><i>RIN1</i></b>	Ras and Rab interactor 1; Ras effector protein. Can affect RAS signalling at different levels. First, by competing with RAF1 protein for binding to activated Ras. Second, by enhancing signaling from ABL1 and ABL2, which regulate cytoskeletal remodelling.
<b><i>RNU4ATAC</i></b>	RNA, U4ATAC small nuclear; Microcephalic osteodysplastic primordial dwarfism, type I.
<b><i>RSPRY1</i></b>	Ring finger- and spry domain-containing protein 1; Spondyloepimetaphyseal dysplasia, Faden-Alkuraya type. Phenotype: progressive spondyloepimetaphyseal dysplasia, short stature, facial dysmorphism, short fourth metatarsals, and intellectual disability.
<b><i>RTTN</i></b>	Rotatin; Microcephaly, short stature, and polymicrogyria with seizures
<b><i>SEMA3E</i></b>	Semaphorin 3E; suspected to cause CHARGE syndrome (short stature is a feature).
<b><i>SF3B4</i></b>	Splicing factor 3b, subunit 4; Nager syndrome is the prototype for a group of disorders collectively referred to as the acrofacial dysostoses (AFDs), which are characterized by malformation of the craniofacial skeleton and the limbs.
<b><i>SHOC2</i></b>	SOC-2 suppressor of clear homolog (C. elegans); Regulatory subunit of protein phosphatase 1 (PP1c) that acts as a M-Ras/MRAS effector and participates in MAPK pathway activation. Noonan-like syndrome with associated growth restriction.
<b><i>SHOX</i></b>	Short stature homeobox; Controls fundamental aspects of growth and development.
<b><i>SLC37A4</i></b>	Solute carrier family 26, member 4; Sodium-independent transporter of chloride and iodide. Glycogen storage disease.
<b><i>SMC1A</i></b>	Structural maintenance of chromosomes 1A; Involved in chromosome cohesion during cell cycle and in DNA repair. Cornelia de Lange syndrome.
<b><i>SOCS2</i></b>	Suppressor of cytokine signaling 2; SOCS family proteins form part of a classical negative feedback system that regulates cytokine signal transduction. SOCS2 appears to be a negative regulator in the growth hormone/IGF1 signaling pathway.
<b><i>SRCAP</i></b>	SNF2-related CREBBP activator protein; Catalytic component of the SRCAP complex which mediates the ATP-dependent exchange of histone H2AZ/H2B dimers for nucleosomal H2A/H2B, leading to transcriptional regulation of selected genes by chromatin remodeling. Defects cause Floating-Harbor syndrome, a rare genetic disorder characterized by proportionate short stature, delayed bone age, delayed speech development,

	and typical facial features.
<b><i>TIMP1</i></b>	TIMP metalloproteinase inhibitor 1; Complexes with metalloproteinases (such as collagenases) and irreversibly inactivates them by binding to their catalytic zinc cofactor. Also mediates erythropoiesis in vitro; but, unlike IL-3, it is species-specific, stimulating the growth and differentiation of only human and murine erythroid progenitors. Known to act on MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13 and MMP-16
<b><i>TMEM126B</i></b>	Transmembrane protein 126B. mitochondrial complex deficiency 1. Associated with a number of clinical features including intrauterine growth retardation, marked growth and developmental delay in humans
<b><i>TRIM37</i></b>	tripartite motif containing 37; E3 ubiquitin-protein ligase. Defects cause Mulibrey nanism.
<b><i>TRMT10A</i></b>	tRNA methyltransferase 10, <i>S. cerevisiae</i> , homolog of, A; Microcephaly, short stature, and impaired glucose metabolism 1.
<b><i>UBR1</i></b>	Ubiquitin protein ligase E3 component n-recognin 1; E3 ubiquitin-protein ligase which is a component of the N-end rule pathway. Binds leucine and is a negative regulator of the leucine-mTOR signaling pathway, thereby controlling cell growth. Defects are associated with Johanson-Blizzard syndrome is an autosomal recessive disorder characterized by poor growth, mental retardation, and variable dysmorphic features, including aplasia or hypoplasia of the nasal alae, abnormal hair patterns or scalp defects, and oligodontia.

153 biological candidate genes which are associated with syndromic growth disorders, skeletal dysplasias, growth plate biology, cell proliferation, DNA replication and repair, code for proteins that interact with partner proteins known to be involved in growth pathways or shown to affect growth in mouse models but without proven link to human linear growth.

Supplementary Table 2. Clinical, biochemical and genetic features in individuals with Silver–Russell syndrome (SRS)

Pt no.	Birth weight SDS	Height SDS	BMI SDS	Target Height SDS	GH basal (µg/L)	GH max (µg/L)	IGF-1 (ng/ml)	IGF-1 SDS	Clinical features	Genetic variants
38	-1.8	-3.7	+0.5	+0.8	12.6	N/D	3.3	-2.8	Classical	H19 hypomethylation
39	-2.3	-4.3	-4.9	-0.5	4.6	12.5	28	-3.4	Classical, blue sclera, high-pitched voice,small face	MatUPD7

H19 hypomethylation, hypomethylation of the imprinting control region 1 (IGF2/H19) in chromosome 11q15; MatUPD7, maternal uniparental disomy of chromosome 7; + parents consanguineous; -, parents not consanguineous. Both patients were previously reported in Storr *et al*, 2015<sup>10</sup>.

**Supplementary Table 3. Genetic variants identified in the GH insensitivity patients by unbiased**

**Analysis 3 (109 variants in 77 candidate genes; n=43 patients)**

Gene	No. variants	No. patients	Gene	No. Variants	No. Patients
<i>ANKRD30A</i>	1	2	<i>MICA</i>	2	4
<i>ANTXR2</i>	1	2	<i>MTCH2</i>	2	3
<i>AQP7*</i>	1	2	<i>MUC17</i>	1	2
<i>ARSD</i>	2	4	<i>MUC2</i>	2	6
<i>BHLHE22</i>	2	3	<i>MUC5AC</i>	1	3
<i>C11orf40</i>	1	3	<i>MUC6*</i>	2	4
<i>C11orf80</i>	1	2	<i>MYO15B</i>	1	3
<i>CAMKK2</i>	1	2	<i>NKAP</i>	1	2
<i>CCDC66</i>	2	4	<i>NLRC5</i>	1	3
<i>CD200R1</i>	1	2	<i>NOTCH4</i>	1	3
<i>CD6</i>	1	3	<i>NPIP8</i>	1	3
<i>CDC42EP1</i>	1	2	<i>OR2T2/OR2T35</i>	1	2
<i>CTBS</i>	1	2	<i>OR4A16</i>	4	2
<i>DFNB59</i>	1	2	<i>OR51B6</i>	1	2
<i>DSPP</i>	2	4	<i>OTOP1</i>	1	2
<i>EI24</i>	1	2	<i>PABPC1</i>	2	4
<i>FAM104B</i>	1	2	<i>PABPC3</i>	1	2
<i>FAM174B</i>	1	2	<i>PODXL</i>	1	3
<i>FAM188B</i>	1	2	<i>PRKRA</i>	1	2
<i>FBXL21</i>	1	3	<i>PRSS3</i>	1	2
<i>FNDC1</i>	1	2	<i>PSG8</i>	1	2
<i>FRG2/FRG2B</i>	1	3	<i>RPL14</i>	1	3
<i>GOLGA6L2</i>	4	5	<i>RXFP2</i>	1	3
<i>GRIA3</i>	1	2	<i>SLC25A5</i>	2	2
<i>GXYLT1</i>	3	4	<i>SLX4</i>	1	3
<i>HGC6.3</i>	1	2	<i>SRRM3</i>	1	3
<i>HLA-DRB1*</i>	4	7	<i>TAS2R19</i>	1	3
<i>IGSF3</i>	1	2	<i>TAS2R31*</i>	1	3
<i>JPH3</i>	1	3	<i>TAS2R43</i>	3	3
<i>KIAA0040</i>	1	2	<i>TEKT4</i>	1	2
<i>KRTAP4-5</i>	1	2	<i>UBXN11</i>	1	2
<i>LCE4A</i>	1	2	<i>USF3</i>	1	2
<i>LGALS8</i>	1	3	<i>ZAN</i>	1	2
<i>LILRA3</i>	1	2	<i>ZFPM1</i>	1	2
<i>LILRB1</i>	1	2	<i>ZNF534</i>	2	3
<i>LOC100129697</i>	2	5	<i>ZNF598</i>	1	2
<i>LOR</i>	1	2	<i>ZNF717</i>	6	5
<i>MAFA</i>	1	3	<i>ZNF91</i>	2	2
<i>MAP3K4</i>	2	2			

Genes were selected if they contained variants which satisfied the following criteria: call quality at least 20, read depth at least 10, allele frequency 0.1% or less in any of the 1000 genomes, ExAC, and all of the NHLBI exomes. Data selected was outside 0.1% of most exonically variable 100 base windows in healthy public genomes and outside 0.1% most exonically variable genes in healthy public genomes (1000 genomes, ExAC). Predicted deleterious changes were defined as disease associated if according to computed ACMG guidelines were classified as pathogenic or likely pathogenic or associated with loss of function of a gene being frameshift, in-frame indel, or start/stop codon change, missense or splice-site change up to 2 bases into the intron. Homozygous or double heterozygous changes were selected if present in novel genes in at least 2 patients but not in the controls. Variants were excluded if the same variant occurred in four or more patients in the likelihood that these were not causal. \* Genes affected in both GH and IGF-1 groups.

**Supplementary Table 4. Univariate logistic regression analysis of age, sex and consanguinity as predictor for a positive genetic diagnosis**

<b>Variable</b>	<b>p value</b>	<b>Odds Ratio</b>	<b>95% CI Lower Limit</b>	<b>95% CI Upper Limit</b>
<b>Age</b>	0.057	0.92	0.84	1.003
<b>Sex</b>	0.25	1.59	0.72	3.48
<b>Consanguinity</b>	<b>0.0001</b>	18.29	6.56	51.06

CI, confidence intervals.